Detection of Pentosidine Cross-Links in Cell-Secreted Decellularized Matrices Using Time Resolved Fluorescence Spectroscopy.

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Public Summary:
Despite a clear correlation between diabetes and diminished fracture repair, the mechanism of impaired bone healing in diabetics remains unclear. The interplay between diabetes and bone formation has been studied using rodent models of diabetes, which suffer from numerous uncontrollable variables, and simplistic collagen films that fail to capture the complexity of the native tissue extracellular matrix. We and others have shown that cell secreted extracellular matrices (ECM) contain numerous instructive cues that drive osteogenic differentiation, angiogenesis, and promote cell survival, suggesting that this platform may provide an improved in vitro model system to study the interplay between the diabetic microenvironment and cell response. Non-enzymatic glycation of long-lived ECM proteins such as type I collagen has been implicated in reduced bone formation. Exposure of collagen to reducing sugars in a diabetic environment catalyzes the formation of advanced glycation end products (AGEs). Sugar molecules react with lysine and arginine residues on collagen to form autofluorescent crosslinks with pentosidine (PENT) as a measurable biomarker of AGEs in collagen. Importantly, there are numerous technical challenges to quantify the formation of non-enzymatic collagen crosslinks that arise due to hyperglycemic conditions. Thus, there is a need for alternative technologies to rapidly and accurately quantify the crosslink formation in engineered materials. This manuscript reports our novel findings describing the application of Time-Resolved Fluorescence Spectroscopy (TRFS) for the detection of PENT in cell-secreted ECM. Upon forming non-enzymatic collagen crosslinks in MSC-secreted matrices, we compared the results of TRFS with HPLC, the gold standard in crosslink quantification, and calculated correlations with a high degree of agreement between the two modalities. After characterizing the composition of the ECM in the presence of ribose-initiated crosslinks, we measured the response of human mesenchymal stem cells (MSCs) to these crosslinked ECMs to test if these conditions induced changes in osteogenic response. Ribose treatment resulted in a 30 nm blue shift in peak fluorescence emission and a significant decrease in average lifetime compared to control matrices (4.4±0.3 ns vs. 3.5±0.09 ns). Evaluation of samples with high performance liquid chromatography (HPLC) confirmed that changes in observed fluorescence were due to PENT accumulation. A strong correlation was found between TRFS parameters and the HPLC measurement of PENT, validating the use of TRFS as an alternative method of PENT detection. Osteoblastic gene expression was significantly reduced in MSCs seeded on ribose ECM at days 7 and 14. However, no significant differences in calcium deposition were detected between control and ribose ECM. These data demonstrate the efficacy of non-destructive fluorescence spectroscopy to examine the formation of non-enzymatic collagen crosslinks within biomimetic culture platforms and showcase one example where an improved biomimetic substrate can be used to probe cell-ECM interactions in the presence of collagen crosslinks.

Scientific Abstract:
Hyperglycemia-mediated, nonenzymatic collagen cross-links such as pentosidine (PENT) can have deleterious effects on cellular interactions with the extracellular matrix (ECM). Present techniques to quantify PENT are limited, motivating the need for improved methods to study the accumulation and contribution of PENT toward diabetic clinical challenges such as impaired bone healing. Current methods for studying PENT are destructive, laborious, and frequently employ oversimplified collagen films that lack the complexity of the native ECM. The primary goal of this study was to evaluate the capacity of time-resolved fluorescence spectroscopy (TRFS) to detect PENT in cell-secreted ECMs possessing enhanced compositional complexity. To demonstrate an application of this method, we assessed the response of human mesenchymal stem cells (MSCs) to cross-linked substrates to explore the role of detected PENT on osteogenic differentiation. We exposed MSC-secreted decellularized matrices (DMs) to 0.66 M ribose for 2 weeks and used TRFS to detect the accumulation of PENT. Ribose treatment resulted in a 30 nm blue shift in peak fluorescence emission and a significant decrease in average lifetime compared to that of control DMs (4.4 +/- 0.3 ns vs 3.5 +/- 0.09 ns). Evaluation of samples with

high performance liquid chromatography (HPLC) confirmed that changes in observed fluorescence were due to PENT accumulation. A strong correlation was found between TRFS parameters and the HPLC measurement of PENT, validating the use of TRFS as an alternative method of PENT detection. Osteoblastic gene expression was significantly reduced in MSCs seeded on ribose DMs at days 7 and 14. However, no significant differences in calcium deposition were detected between control and ribose DMs. These data demonstrate the efficacy of nondestructive fluorescence spectroscopy to examine the formation of nonenzymatic collagen cross-links within biomimetic culture platforms and showcase one example where an improved biomimetic substrate can be used to probe cell-ECM interactions in the presence of collagen cross-links.
